Quantification of Arbuscular Mycorrhizal Fungal Biomass in a Changing Global Environment

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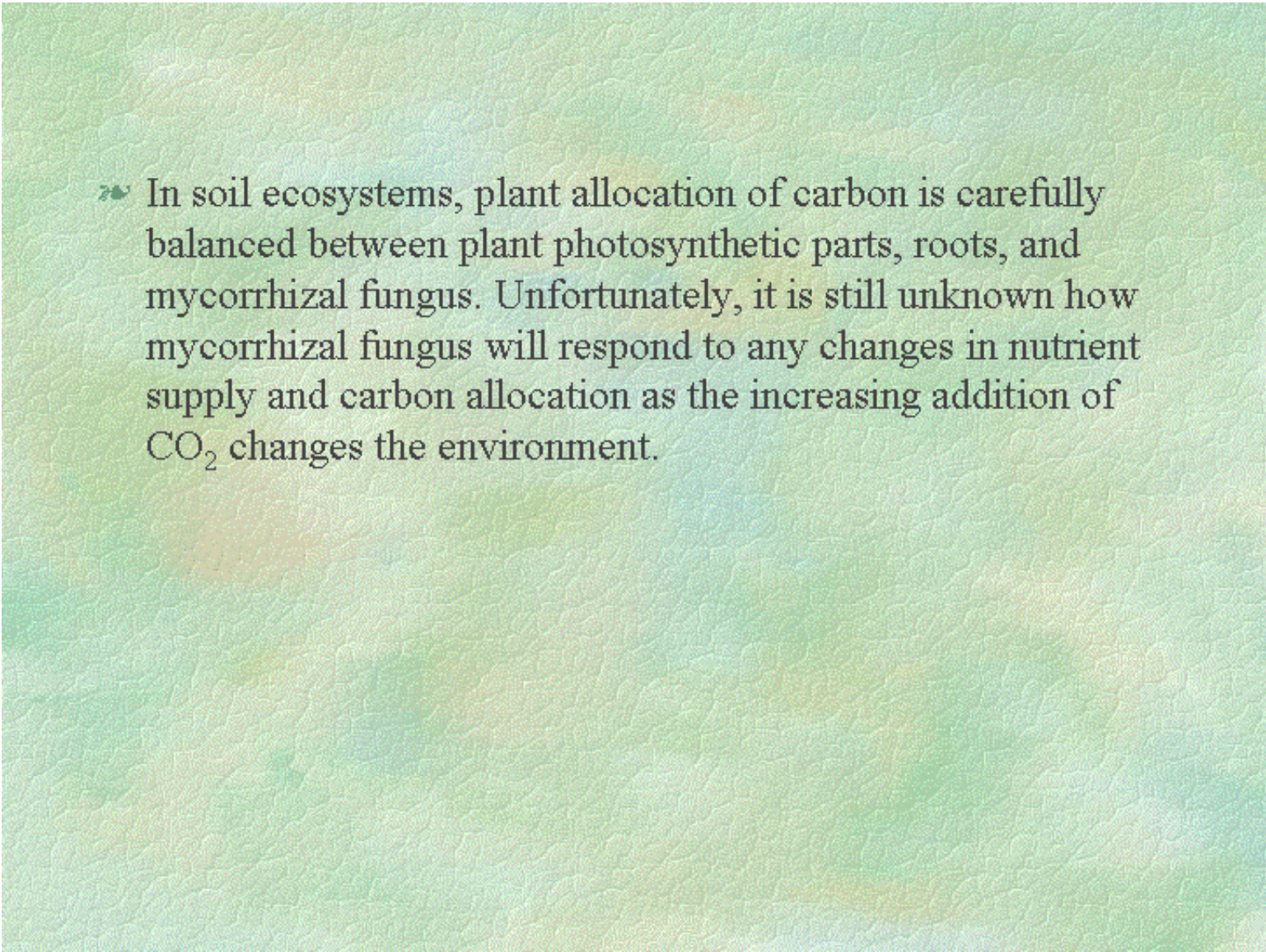
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Abstract

The arbuscular mycorrhizal fungal association with plants has been suggested as an important pool for carbon sequestration. As climate changes and levels of CO_2 rise, it is unknown how carbon levels will change in the soil, including the levels of carbon input by mycorrhizal fungi. Methods for quantifying carbon are difficult. Chitin, the predominant form of carbon in fungal cell walls, can be detected through a colorimetric assay of colonized root tissue. The colorimetric methods involve depolymerization and deacetylization of chitin in root tissue by concentrated KOH to form chitosan, which is deaminated and further broken down into glucosamines.

When MBTH is added, the glucosamines form a complex that react with ferric chloride to make a blue solution. When compared to a standard curve, the spectrophotometric absorbance of the resulting solution will give chitin levels. We found that the chitin levels have a very high correlation to fungal biomass. This indicates that the chitin determination method is a valid method for measuring mycorrhizal biomass, and can be used in future works to measure carbon allocation in fungus.

Fungus and Global Change

- Arbuscular fungi are found in close symbiotic association with plant roots. These mycorrhizal fungi are an essential part of soil systems, aiding in the nutrient uptake of most terrestrial plants, and in return accepting generous carbon donations from their host plants. In this manner, carbon is quickly transported from plants to fungi, and from fungi to the rest of the soil ecosystem. The soil acts as a large carbon reservoir, as well as a net carbon sink.

- The soil ecosystem is relatively unexplored territory; not much is known about the complexities of this diverse and tremendously important ecosystem, especially under a changing environment. What will happen to soil as a carbon sink as CO_2 levels rise, for example, is still a complicated mystery.

In soil ecosystems, plant allocation of carbon is carefully balanced between plant photosynthetic parts, roots, and mycorrhizal fungus. Unfortunately, it is still unknown how mycorrhizal fungus will respond to any changes in nutrient supply and carbon allocation as the increasing addition of CO_2 changes the environment.

Carbon in Fungus

- In order to understand carbon allocation to mycorrhizal fungus and how it will change as the environment changes, we need a method to accurately determine mycorrhizal biomass. Fungal biomass is difficult to assess, especially for endomycorrhizal plants, and is typically inferred from percent colonization of roots. However, colonization data does not give carbon values in fungal tissue.

- Fungal biomass, a more direct measure of the amount of fungal carbon, can be calculated from biovolume measurements by determining the volume of fungus within root tissue and converting to biomass.

Figure 1. Root demonstrating both intraradical and extraradical Hyphal growth for the arbuscular mycorrhizal fungus.

Biomass Calculations through Chitin Determination

* Chitin is a polymer of glucosamines found in fungal cell walls (it is also found in the external skeletons of insects and arthropods). Measuring chitin content in root tissues is a direct way to measure carbon allocation in endomycorrhizal fungus. Unfortunately, the chitin in parasitic fungi and other pathogenic organisms present in the plant root will also be measured, and therefore the measurement of chitin for mycorrhizal fungus biomass determination cannot be readily utilized in natural systems. Chitin levels, however, can be used to calibrate the fungal biomass from root tissue taken in the field, helping to better determine the carbon allocation in mycorrhizal fungus.

Fig 2. Calculation of Intraradical Fungus Biomass

Fungus biovolume (V_{if})

$$V_{if} = r_0^2 * L_c * \pi * k_f$$

Fungus biomass (B_{if})

$$B_{if} = S_{if} * d_{if} * V_{if}$$

r = root radius

r_0 = sqrt(W_f/L* π)

W_f = root fresh weight

L = fibrous root length

L_c = colonized root length

k_f = fraction colonized root length that is fungus (0.06)

k_s = proportion of root that is cortex

V_{if} = fungus biovolume

d_{if} = wet specific gravity (1.1 g cm^{-3})

S_{if} = dry matter content (0.25 g cm^{-3})

Chitin Determination Methods

- **Chitin is converted to chitosan**: 10 mg alliquots of finely ground representative samples of root tissue are autoclaved at 120° with 4 ml of HCl in 15 ml centrifuge tubes.

- **Chitosan is precipitated** in subsequent cold ethanol and water washes and centrifuged at 1500g's for 10 minutes.

- **Chitosan is deacetylated** and partially deaminated with 1.5 ml each of 5% NaNO2, 5% KHSO4, and 0.5 ml of 12.5% NH_4SO_3NH2.

- **Resulting glucosamine residues treated** with 0.5 ml of 0.5% 3-methyl-2-benzothiazolone hydrazone (MBTH), which forms a blue complex that reacts with 0.5 ml of 0.5% FeCl3. The absorbance of the resulting blue solution is measured in a spectrophotometer at 650 nm and compared to a standard curve of absorbance values for known concentrations of chitin. This gives the amount of fungal biomass chitin.

Conclusions

✒ We found a direct relationship between the calculated fungal biomass and chitin level within a root system of the grass *Andropogon gerardii* (Figure 3). This suggests that through further parameterization of the calculated fungal biomass procedure (Figure 2) that it should be possible to improve the allometric relationship between fungal biovolume and chitin.

Future work

- Future research will be directed at improving upon parameterization of the fungal and root structures used in the calculation of the fungal biovolume. In addition, further research will be needed to determine the relevance of the approach used in this study to a range of root morphologies and containing different arbuscular mycorrhizal fungal species.

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